FIG. 1

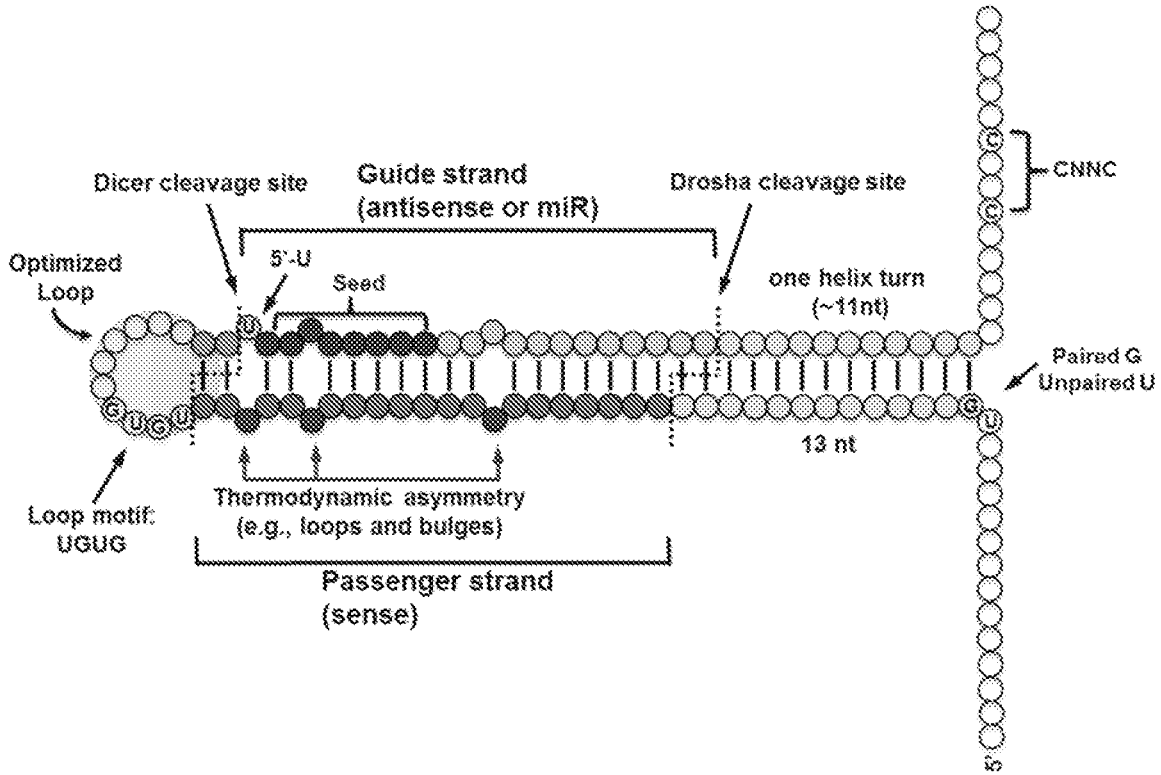

Dicer cleavage site
Guide strand
(antisense or miR)
Drosha cleavage site
CNNC
Optimized
Loop
5'-U
Seed
one helix turn
(~11nt)
Paired G
Unpaired U
13 nt
Loop motif:
UGUG
Thermodynamic asymmetry
(e.g., loops and bulges)
Passenger strand
(sense)
5'

FIG. 2

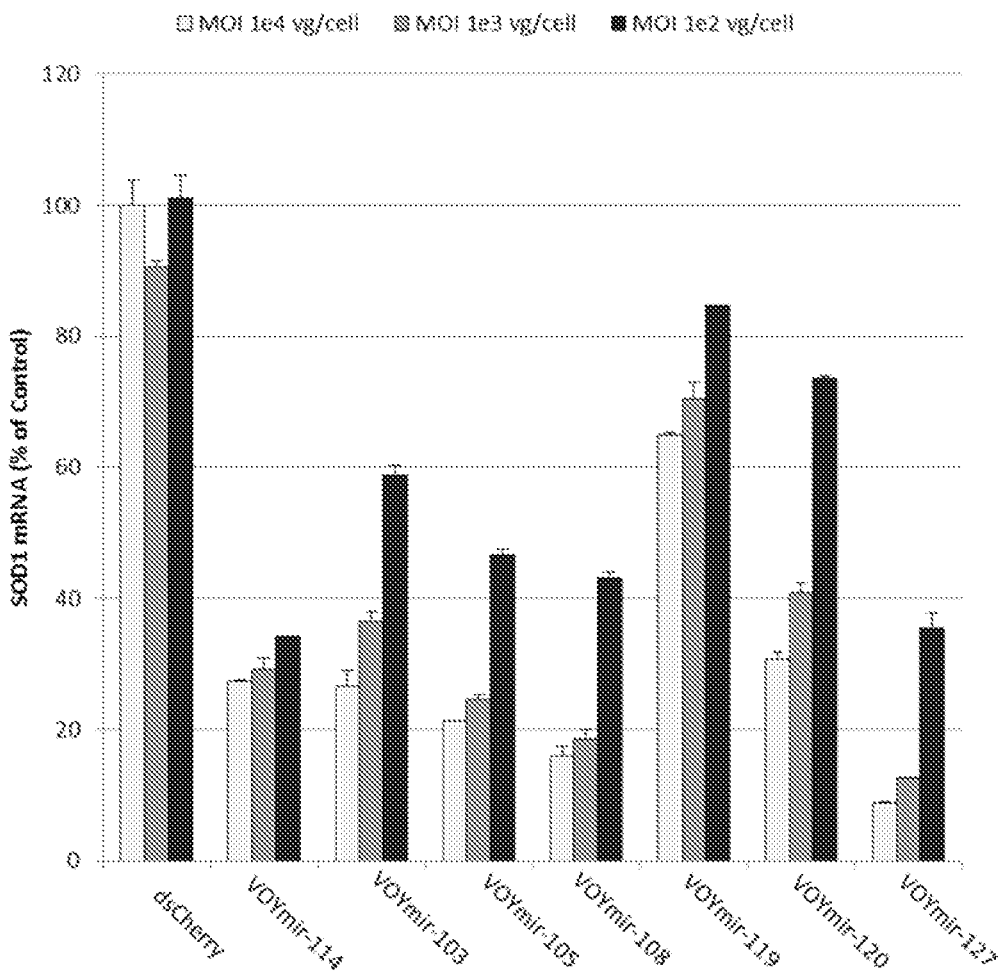

MOI 1e4 vg/cell
MOI 1e3 vg/cell
MOI 1e2 vg/cell
SOD1 mRNA (% of Control)
120
100
80
60
40
20
0
dsCherry
VOYmir-114
VOYmir-103
VOYmir-105
VOYmir-108
VOYmir-119
VOYmir-120
VOYmir-127

FIG. 3

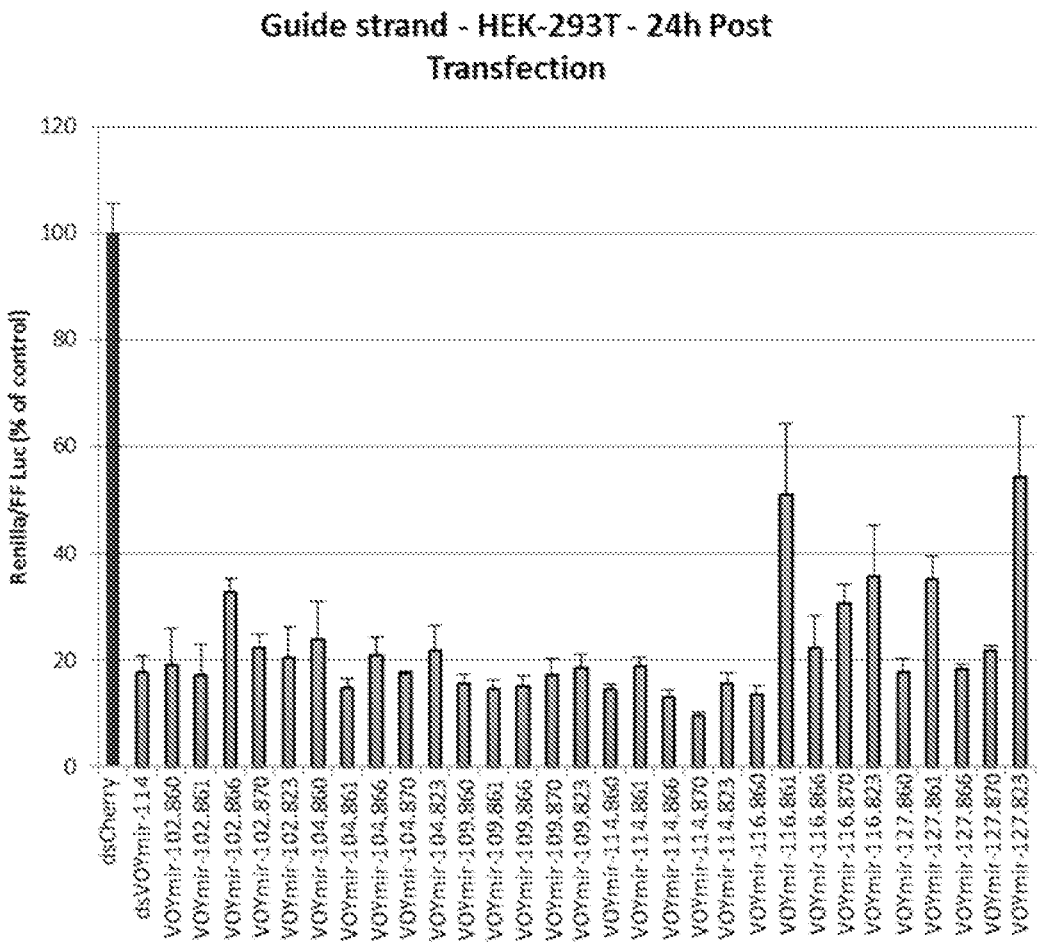

Guide strand - HEK-293T - 24h Post Transfection
Renilla/FF Luc (% of control)
120
100
80
60
40
20
0
dsCherry
dsVOYmir-114
VOYmir-102.860
VOYmir-102.861
VOYmir-102.866
VOYmir-102.870
VOYmir-102.823
VOYmir-104.860
VOYmir-104.861
VOYmir-104.866
VOYmir-104.870
VOYmir-104.823
VOYmir-109.860
VOYmir-109.861
VOYmir-109.866
VOYmir-109.870
VOYmir-109.823
VOYmir-114.860
VOYmir-114.861
VOYmir-114.866
VOYmir-114.870
VOYmir-114.823
VOYmir-116.860
VOYmir-116.861
VOYmir-116.866
VOYmir-116.870
VOYmir-116.823
VOYmir-127.860
VOYmir-127.861
VOYmir-127.866
VOYmir-127.870
VOYmir-127.823

FIG. 4

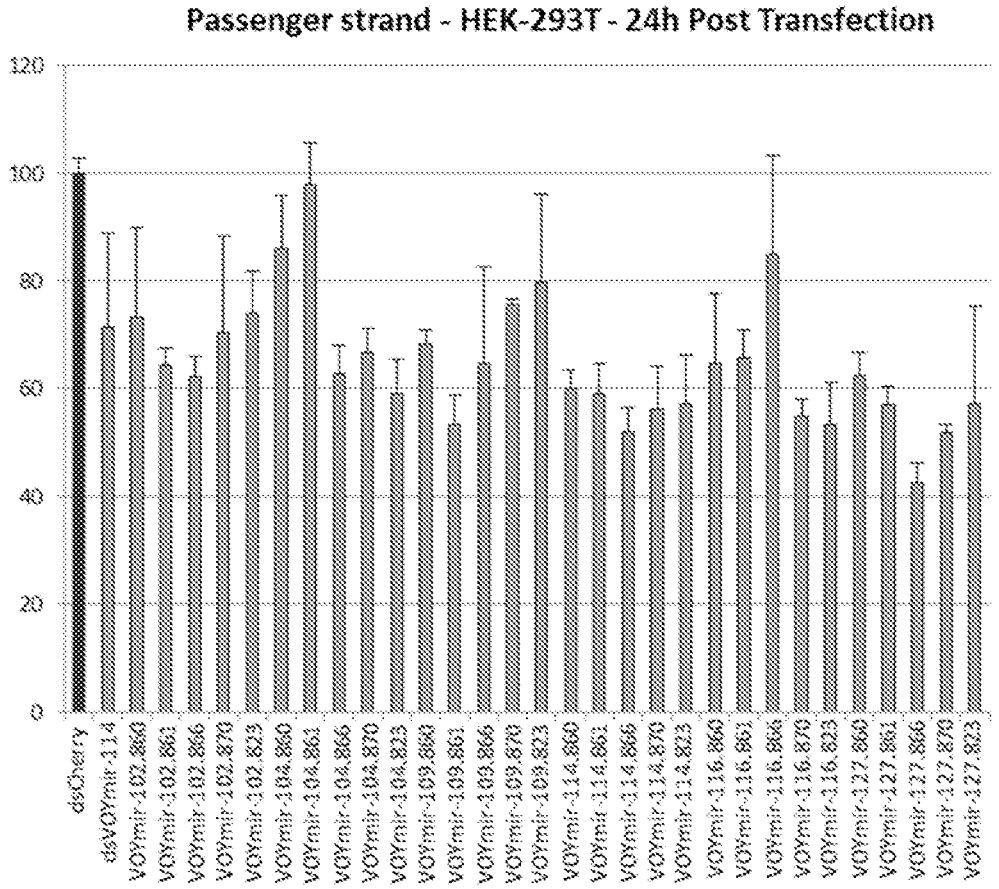

Passenger strand - HEK-293T - 24h Post Transfection
120
100
80
60
40
20
0
dsCherry
dsVOYmir-114
VOYmir-102.860
VOYmir-102.861
VOYmir-102.866
VOYmir-102.870
VOYmir-102.823
VOYmir-104.860
VOYmir-104.861
VOYmir-104.866
VOYmir-104.870
VOYmir-104.823
VOYmir-109.860
VOYmir-109.861
VOYmir-109.866
VOYmir-109.870
VOYmir-109.823
VOYmir-114.860
VOYmir-114.861
VOYmir-114.866
VOYmir-114.870
VOYmir-114.823
VOYmir-116.860
VOYmir-116.861
VOYmir-116.866
VOYmir-116.870
VOYmir-116.823
VOYmir-127.860
VOYmir-127.861
VOYmir-127.866
VOYmir-127.870
VOYmir-127.823

FIG. 5

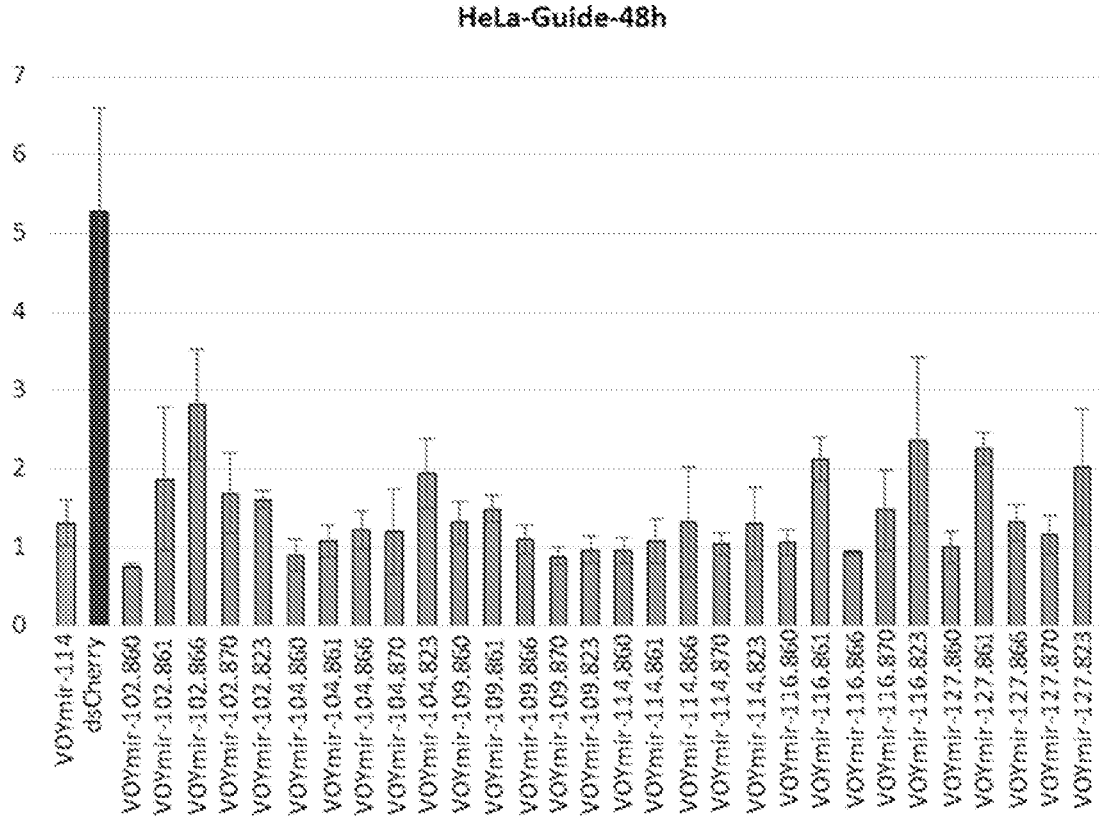

HeLa-Guide-48h
7
6
5
4
3
2
1
0
VOYmir-114
dsCherry
VOYmir-102.860
VOYmir-102.861
VOYmir-102.866
VOYmir-102.870
VOYmir-102.823
VOYmir-104.860
VOYmir-104.861
VOYmir-104.866
VOYmir-104.870
VOYmir-104.823
VOYmir-109.860
VOYmir-109.861
VOYmir-109.866
VOYmir-109.870
VOYmir-109.823
VOYmir-114.860
VOYmir-114.861
VOYmir-114.866
VOYmir-114.870
VOYmir-114.823
VOYmir-116.860
VOYmir-116.861
VOYmir-116.866
VOYmir-116.870
VOYmir-116.823
VOYmir-127.860
VOYmir-127.861
VOYmir-127.866
VOYmir-127.870
VOYmir-127.823

FIG. 6

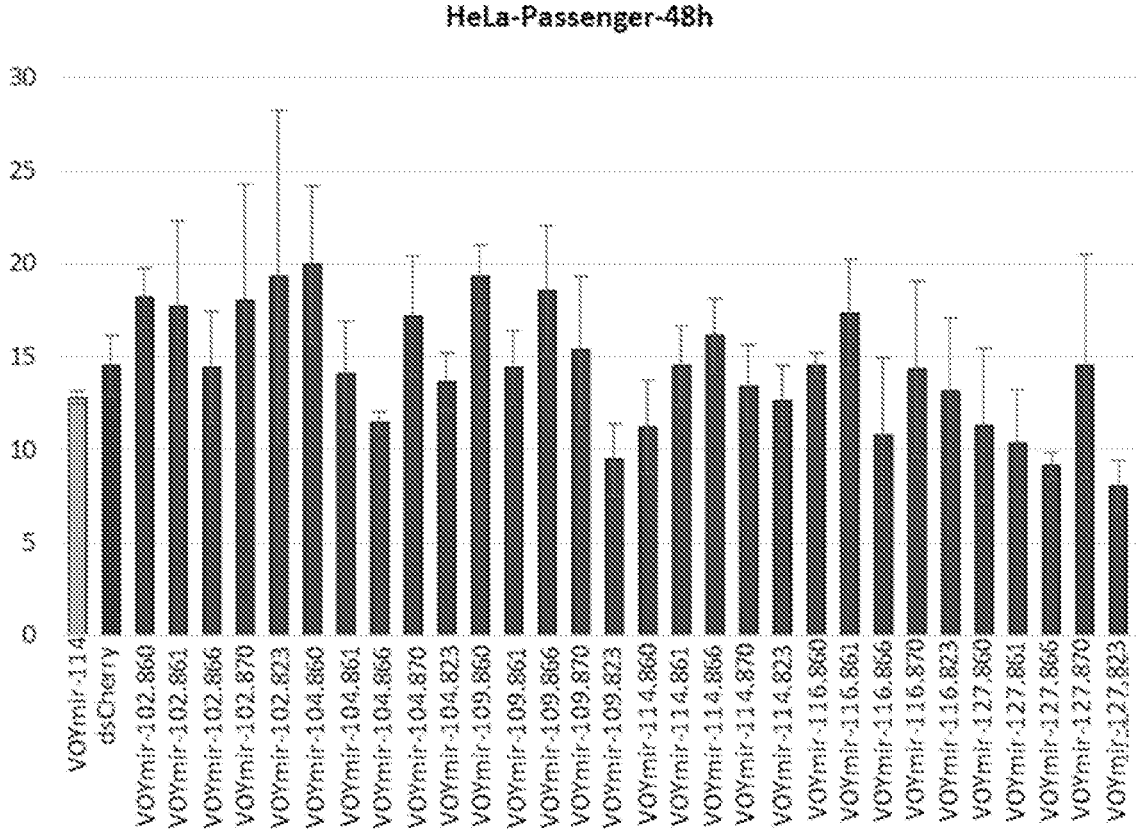

HeLa-Passenger-48h
30
25
20
15
10
5
0
VOYmir-114
dsCherry
VOYmir-102.860
VOYmir-102.861
VOYmir-102.866
VOYmir-102.870
VOYmir-102.823
VOYmir-104.860
VOYmir-104.861
VOYmir-104.866
VOYmir-104.870
VOYmir-104.823
VOYmir-109.860
VOYmir-109.861
VOYmir-109.866
VOYmir-109.870
VOYmir-109.823
VOYmir-114.860
VOYmir-114.861
VOYmir-114.866
VOYmir-114.870
VOYmir-114.823
VOYmir-116.860
VOYmir-116.861
VOYmir-116.866
VOYmir-116.870
VOYmir-116.823
VOYmir-127.860
VOYmir-127.861
VOYmir-127.866
VOYmir-127.870
VOYmir-127.823

FIG. 7

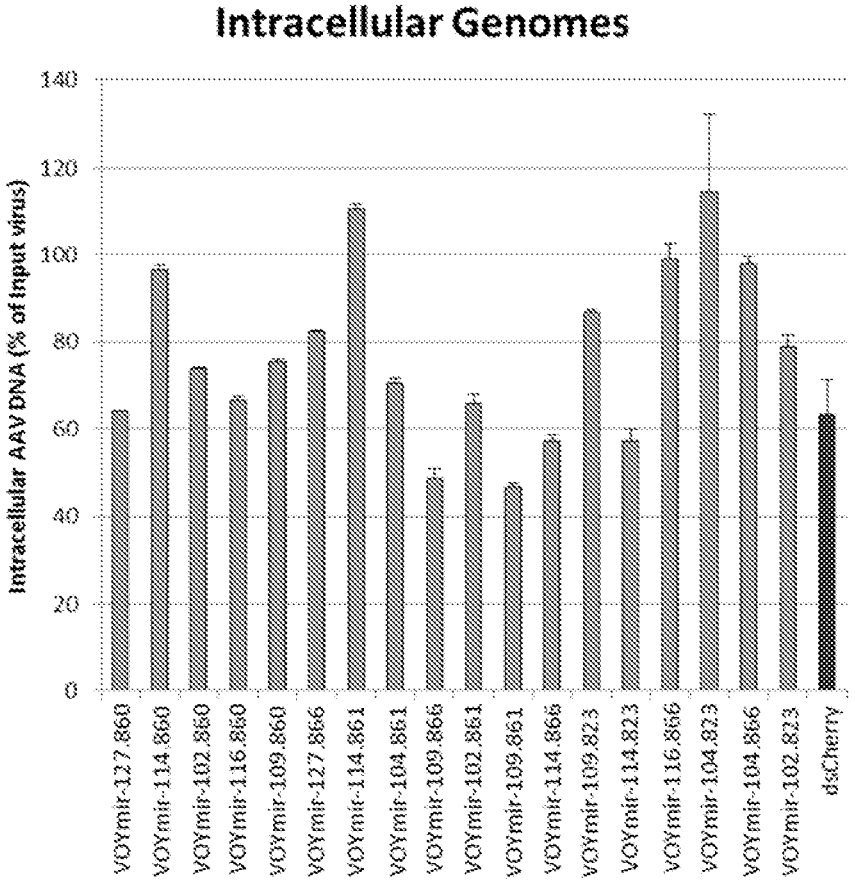

Intracellular Genomes
Intracellular AAV DNA (% of input virus)
140
120
100
80
60
40
20
0
VOYmir-127.860
VOYmir-114.860
VOYmir-102.860
VOYmir-116.860
VOYmir-109.860
VOYmir-127.866
VOYmir-114.861
VOYmir-104.861
VOYmir-109.866
VOYmir-102.861
VOYmir-109.861
VOYmir-114.866
VOYmir-109.823
VOYmir-114.823
VOYmir-116.866
VOYmir-104.823
VOYmir-104.866
VOYmir-102.823
dsCherry

FIG. 8

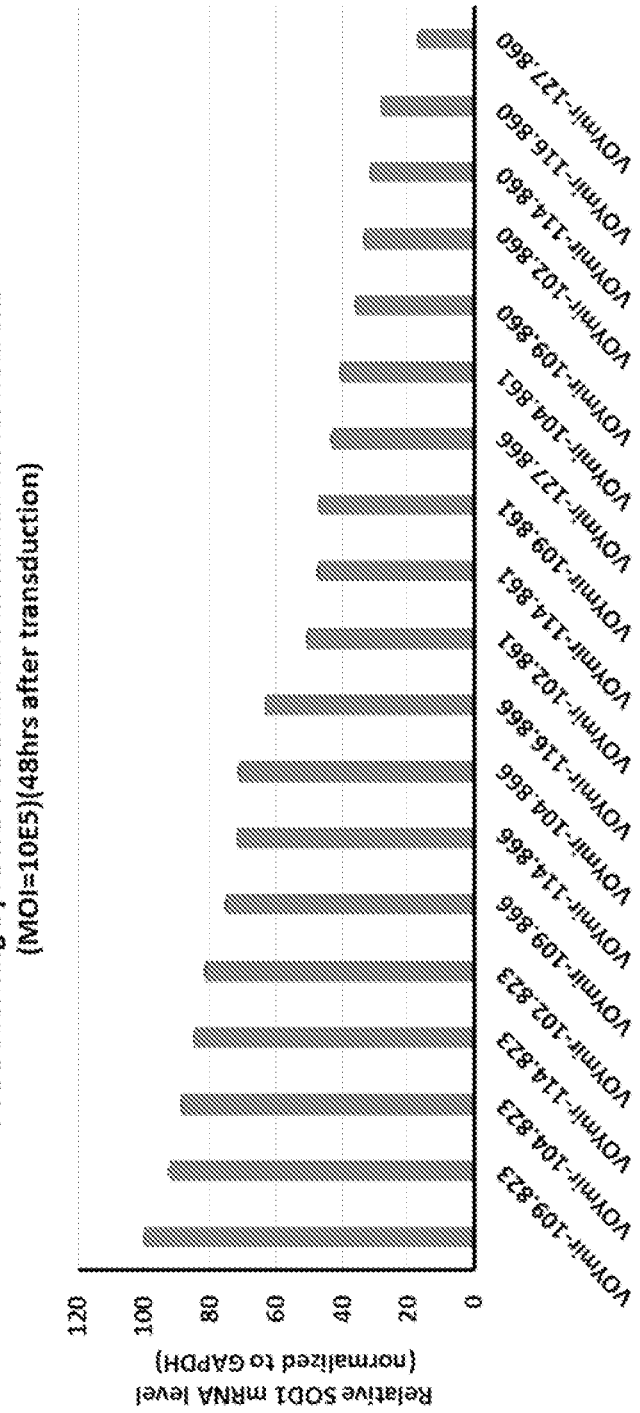

SOD1 silencing by AAV2-SOD1 miRNAs in human motor neurons
(MOI=10E5)(48hrs after transduction)
Relative SOD1 mRNA level
(normalized to GAPDH)
120
100
80
60
40
20
0
VOVmir-109.823
VOVmir-104.823
VOVmir-114.823
VOVmir-102.823
VOVmir-109.866
VOVmir-114.866
VOVmir-104.866
VOVmir-116.866
VOVmir-102.861
VOVmir-114.861
VOVmir-109.861
VOVmir-127.866
VOVmir-104.861
VOVmir-109.860
VOVmir-102.860
VOVmir-114.860
VOVmir-116.860
VOVmir-127.860

FIG. 9

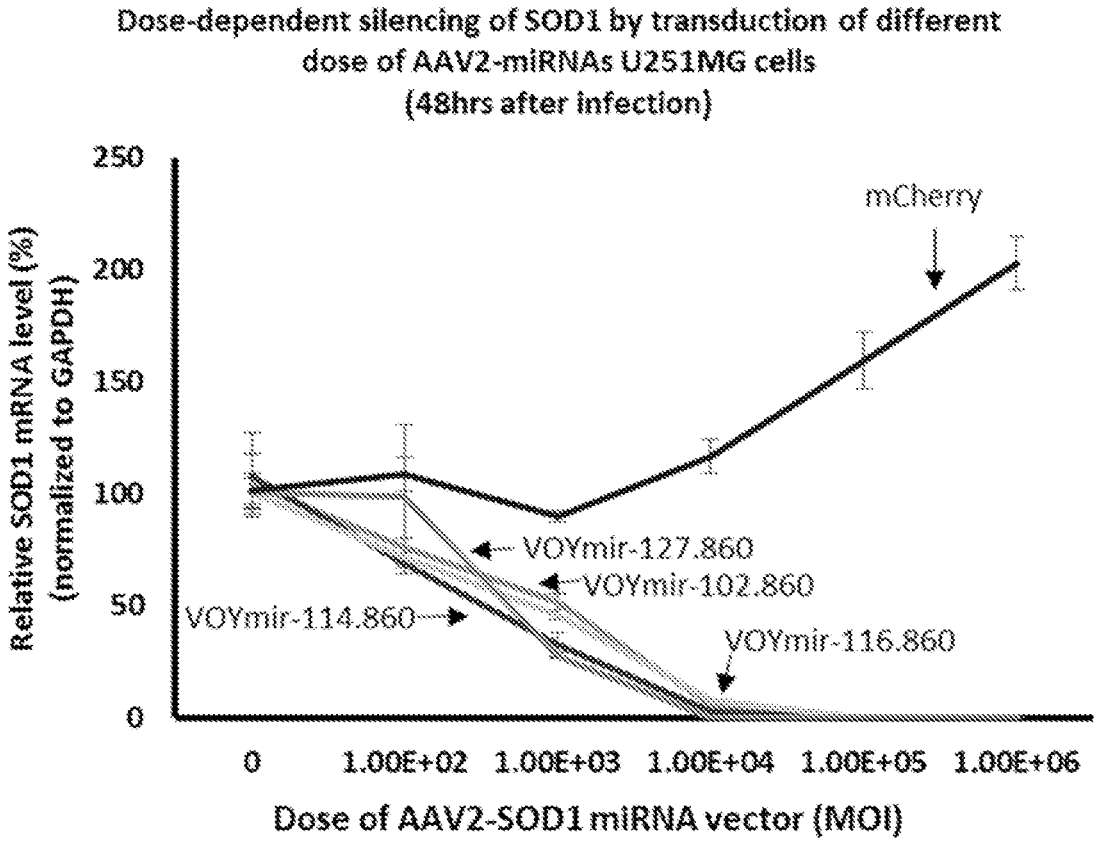

Dose-dependent silencing of SOD1 by transduction of different dose of AAV2-miRNAs U251MG cells (48hrs after infection)
Relative SOD1 mRNA level (%) (normalized to GAPDH)
250
200
150
100
50
0
mCherry
VOYmir-127.860
VOYmir-102.860
VOYmir-114.860
VOYmir-116.860
0
1.00E+02
1.00E+03
1.00E+04
1.00E+05
1.00E+06
Dose of AAV2-SOD1 miRNA vector (MOI)

FIG. 10

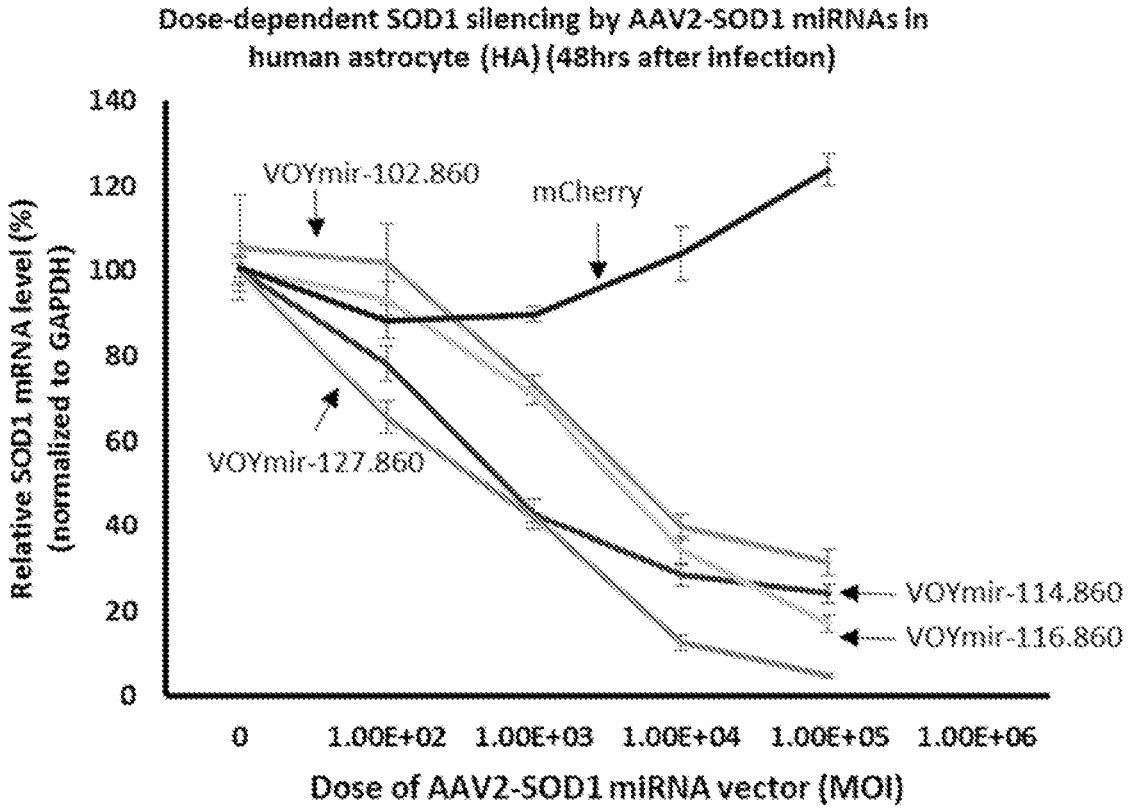

Dose-dependent SOD1 silencing by AAV2-SOD1 miRNAs in human astrocyte (HA) (48hrs after infection)
Relative SOD1 mRNA level (%) (normalized to GAPDH)
140
120
100
80
60
40
20
0
VOYmir-102.860
mCherry
VOYmir-127.860
VOYmir-114.860
VOYmir-116.860
0
1.00E+02
1.00E+03
1.00E+04
1.00E+05
1.00E+06
Dose of AAV2-SOD1 miRNA vector (MOI)

FIG. 11

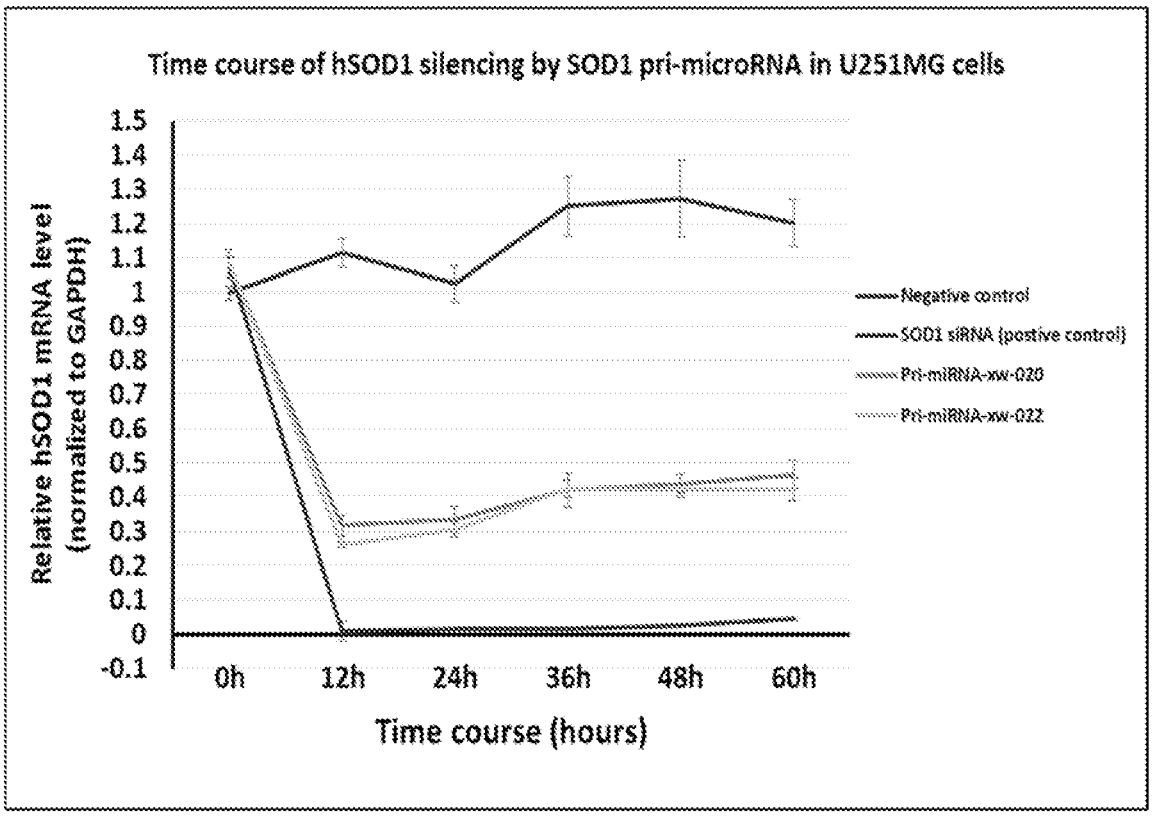

Time course of hSOD1 silencing by SOD1 pri-microRNA in U251MG cells
Relative hSOD1 mRNA level (normalized to GAPDH)
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
0h
12h
24h
36h
48h
60h
Time course (hours)
Negative control
SOD1 siRNA (postive control)
Pri-miRNA-xw-020
Pri-miRNA-xw-022

FIG. 12A

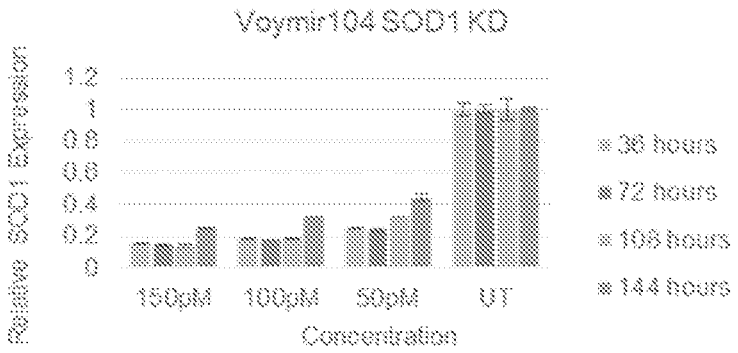


FIG. 12B

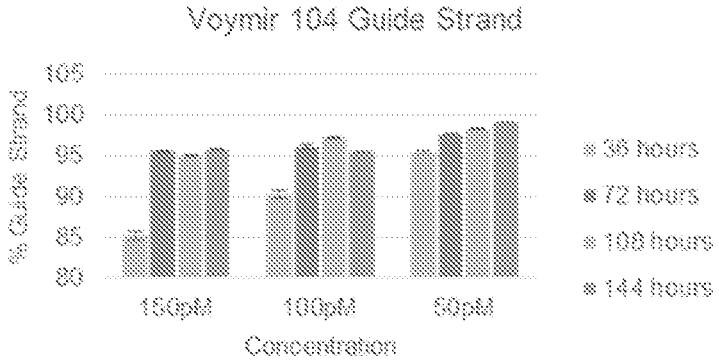


FIG. 12C

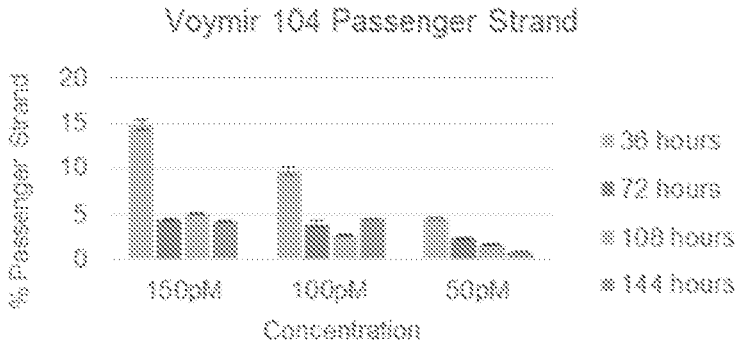
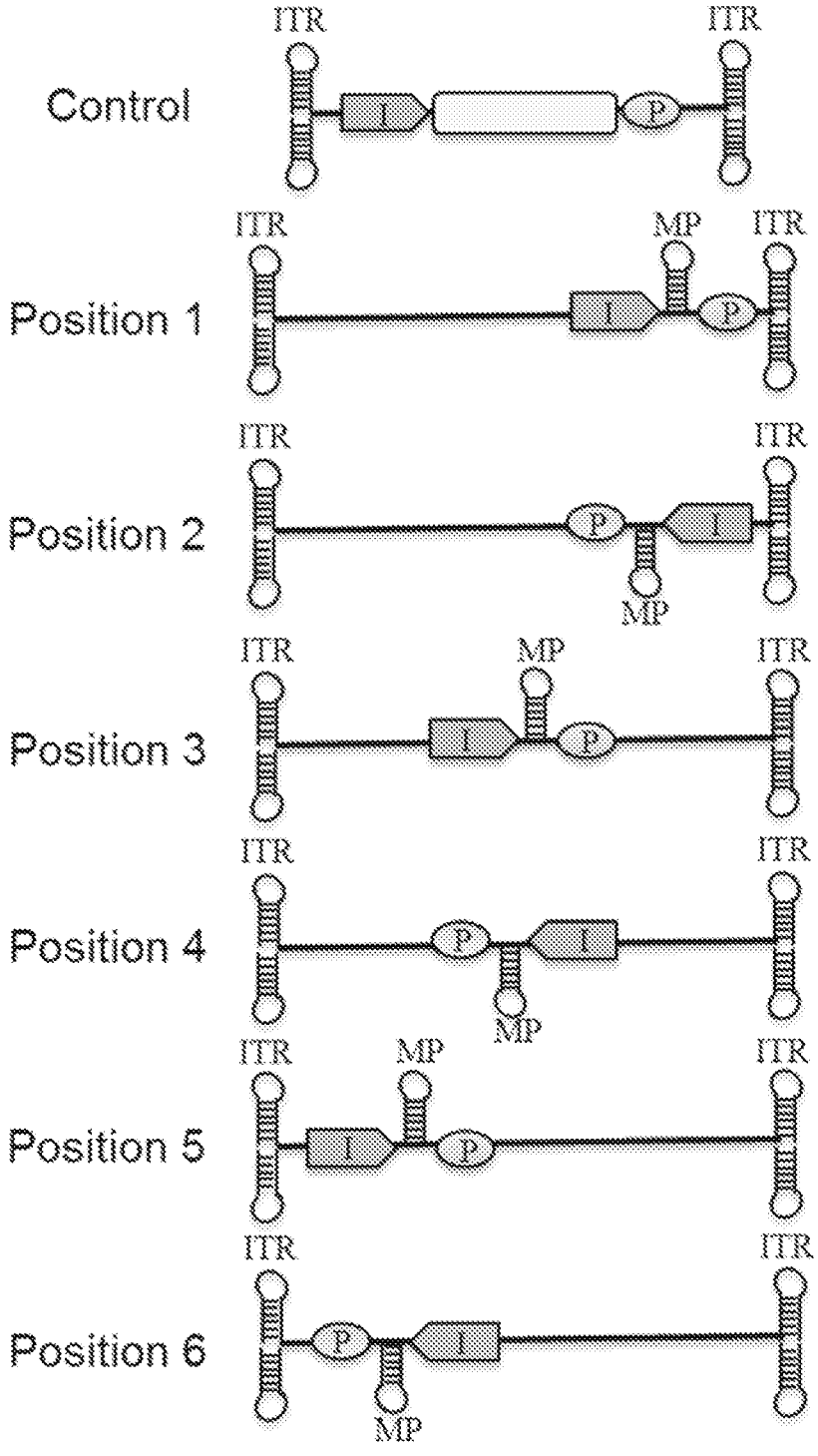


FIG. 13


Control
ITR
I
P
ITR
Position 1
ITR
MP
ITR
Position 2
ITR
ITR
Position 3
ITR
MP
ITR
Position 4
ITR
ITR
Position 5
ITR
MP
ITR
Position 6
ITR
ITR